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Effects of Hybridization on Pigmentation in Fishes of the Genus *Xiphophorus*

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(Plates I-VIII)

CONTENTS

I. INTRODUCTION

THIS PAPER is dedicated to Myron Gordon. The dedication is particularly appropriate for a number of reasons. Most of the hybrids treated herein were produced under Dr. Gordon's direction, and publication of this work was one of many projects left unfinished by his untimely death in 1959. Dr. Gordon had caught the parental stock from which the crosses were made, founded the unique Genetics Laboratory of the New York Aquarium in which they were made and, most important of all, provided the rationale under which their existence and study assumed significance. It is especially fitting that the paper appears in the scientific journal of the New York Zoological Society; Dr. Gordon was intimately associated with the Society for the major part of his career and numerous scientific reports by him and his associates were published in *Zoologica* over the years. Finally, deep personal feelings add another dimension to the dedication. Myron Gordon was a dear friend as well as mentor. It was he who suggested the pigmentation of hybrids as a subject for a doctoral thesis and made available the fishes and facilities of the Genetics Laboratory.

Myron Gordon first recognized the remarkable combination of features that makes the fishes of the genus *Xiphophorus* so worthy of study (Atz & Rosen, 1959). Not the least of these is their ability to hybridize with one another. In fact, it was the melanotic hybrids of *X. maculatus–X. hellerii* that first brought these fishes to the attention of biologists and medical men (Atz, 1941). Because they have been more readily available for experimentation than the other species of *Xiphophorus*, because their hybrids often develop melanoma and because *X. maculatus* shows an unusual but clear-cut sex-linkage, these two species have been the subject of many more investigations than any of their congeners. Dr. Gordon's broad approach to the problems of comparative oncolgy, however, included a study of all the then known species of

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[2] From a dissertation submitted to the Graduate School of Arts and Science, New York University, New York, N. Y., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Xiphophorus and their hybrids.[3] As can be seen in Table I, nearly two-thirds of the 28 crosses recorded up to the present were first made by Dr. Gordon (see the papers by Gordon, Gordon *et al.* and Rosen).

The author wishes to thank Dr. Donn E. Rosen for invaluable help received during many long and spirited discussions. Dr. Klaus D. Kallman, Research Associate in Genetics of the New York Aquarium, who succeeded Dr. Gordon in direction of the Genetics Laboratory, also provided valuable assistance, including a critical reading of the manuscript, and this is gratefully acknowledged. Special thanks are due the American Museum of Natural History, in particular the Department of Birds, for their most generous provision of space and facilities.

II. Materials and Methods

The hybrid fishes upon which this study is based have been produced over a period of more than 25 years, but the great majority of them are the result of crosses set up since 1939 in the Genetics Laboratory of the New York Aquarium. Almost all of the stocks of fishes that have been used were derived from specimens collected alive in their native Mexico or British Honduras, thus insuring purity of ancestry, for domesticated fish, obtained from pet stores or commercial breeders, almost invariably have a hybrid somewhere among their progenitors.

The following is a list of the strains used in the present studies, the geographical area from which they were taken, and the expedition responsible for collecting the foundation specimens:

Xiphophorus couchianus (Girard, 1859)[4]

Rio Santa Catarina, Nuevo Leon (1939) — Gordon, Atz, Evelyn Gordon; (1958)—Gordon, Evelyn Gordon.

Xiphophorus variatus xiphidium (Gordon, 1932)

Rio Purificacion, Tamaulipas (1939)—Gordon, Atz, Evelyn Gordon.

Rio Santa Engracia, Tamaulipas (1958) — Gordon, Evelyn Gordon.

Xiphophorus variatus variatus (Meek, 1904)

Rio Axtla, San Luis Potosi (1939)—Gordon, Atz, Evelyn Gordon.

Xiphophorus variatus evelynae Rosen, 1960

Rio Necaxa, Puebla (1957)—Rosen, Malcolm Gordon, Gordon.

Xiphophorus montezumae montezumae Jordan & Snyder, 1900

Rio Salto, San Luis Potosi (1957)—Rosen, Malcolm Gordon, Gordon.

Xiphophorus montezumae cortezi Rosen, 1960

Rio Axtla, San Luis Potosi (1939)—Gordon, Atz, Evelyn Gordon.

Xiphophorus pygmaeus pygmaeus Hubbs & Gordon, 1943

Rio Axtla, San Luis Potosi (1940)—New York Aquarium Expedition to La Cueva Chica, C. M. Breder, Jr., leader.

Xiphophorus maculatus (Guenther, 1866)

8A—Domesticated, white, spotted strain (August 7, 1939)—Matsuno, New York, N. Y.

23 & 30—Rio Jamapa, Veracruz (1939)—Gordon, Atz, Evelyn Gordon.

Gp—Rio Grijalva, Tabasco (1952)—Gordon.

Bp—Belize River, British Honduras (1949)—Gordon, Fairweather.

Xiphophorus hellerii strigatus Regan, 1907

3B—Arroyo Zacatispan, Oaxaca (1939)—Gordon, Atz, Evelyn Gordon.

124—Domesticated strain.

Xiphophorus hellerii guentheri Jordon & Evermann, 1896

Gx—Rio Grijalva, Tabasco (1952)—Gordon.

Bx—Belize River, British Honduras (1949)—Gordon, Fairweather.

Xiphophorus hellerii

hx—Domesticated, albino strain.

A description of the laboratory in which these viviparous, tropical, freshwater fishes have been maintained and the care that is given them may be found in Gordon (1950c).

A total of 3,000 hybrids was involved in the present study. Most of the specimens were fixed and stored in formalin, some in ethyl alcohol. In addition to the hybrids, preserved examples of the strains from which they had been derived were examined, as well as numerous fish caught and preserved in the wild. The latter are now part of the collections of the Museum of Zool-

[3] In accord with this approach, the recent discovery of two new species, *X. clemenciae* and *X. milleri*, and four new subspecies (Rosen, 1960) opens up a wide field of comparative genetics.

[4] With the exception of the spelling of *hellerii*, these scientific names are the same as the ones used by Rosen (1960) in his comprehensive revision of the teleost genus *Xiphophorus* (Family Poeciliidae, Order Cyprinodontiformes). The orthographic change is required by the International Code of Zoological Nomenclature adopted by the XV International Congress of Zoology and published in 1961.

TABLE I. EARLIEST REFERENCES TO HYBRIDIZATION OF FISHES OF THE GENUS *Xiphophorus*[1]

Female	*couchianus*	*variatus xiphidium*	*variatus variatus*	Male *maculatus*	*montezumae cortezi*	*pygmaeus pygmaeus*	*hellerii*
couchianus			Rosen (1960) ●	Gordon (1941) ●			
variatus xiphidium			Nigrelli & Gordon (1951) Rosen (1960) ●	Kosswig (1935a) ●	Kosswig (1959)		
variatus variatus	Rosen (1960) ●	Rosen (1960) ●		Bellamy (1936) Gordon & Smith (1938)		Gordon (1953) ●	Gordon (1941)[2]
maculatus	Gordon (1933) Reed, Gordon & Lansing (1933) Gordon & Smith (1938) ●	Gordon (1933) Kosswig (1935a) Gordon & Smith (1938) ●	Gordon (1933) Kosswig (1935a,b) Bellamy (1936) Gordon & Smith (1938) ●		Gordon (1941) Gordon (1951b) ●	Gordon (1941) Gordon (1951b)[2]	Lösslein (1912)[3] Hafner (1912)[3]
montezumae cortezi	Rosen (1960) ●	Kosswig (1959) ●	Gordon (1953) ●	Gordon (1941) ●			Kosswig (1936) ●
pygmaeus pygmaeus		Gordon (1941) ●	Gordon (1953) ●		Gordon (1953) ●		
hellerii	Rosen (1960) ●	Kosswig (1935a, 1936)	Rust (1941) Gordon (1941)[2]	Gerschler (1914)[3] ●			

[1] The two or three subspecies of *X. hellerii* involved in various crosses have not been distinguished, partly because of the difficulty of identifying what form was used. Specimens of hybrids from the combinations marked with a dot (●) have been studied for this paper.

[2] No record or other evidence of this cross could be found in the Genetics Laboratory after the death of Dr. Myron Gordon.

[3] According to the historical review presented by Gordon (1931b).

ogy of the University of Michigan, Ann Arbor, Michigan (Rosen, 1960).

For practical reasons, we have had to confine our attention to patterns visible on the surface of the fish and also to melanic pigmentation, because the latter resists fading in ethyl alcohol, formaldehyde or glycerine — in contrast to the red patterns that occur in these fishes, for example. Two special techniques were used to facilitate the study of the morphology of pigment cells and patterns. Selected specimens were dehydrated in absolute alcohol and cleared in methyl salicylate (synthetic oil of wintergreen), as described by Gordon (1931a). The skins of others were dehydrated, cleared in xylene and mounted on slides in Permount.

The photographs of living and preserved fish were taken by Sam Dunton, Photographer of the New York Zoological Society, while those of cleared specimens and skins were made by Dr. Ross F. Nigrelli, Pathologist of the New York Aquarium.

III. Results

From both a genetic and morphological point of view, the pigmentary patterns of the fishes of the genus *Xiphophorus* may be divided into (1) those shared by all adult members of the form, or sometimes all adult members of one sex of the form, and (2) those found in some individuals but not in others. The latter comprise the polymorphic elements of the pigmentary system. They are made up of either micromelanophores or macromelanophores and are genetically controlled in most instances by single major genes. In contrast, the monomorphic or non-polymorphic patterns are composed of micromelanophores and scale melanophores and, as far as known, are controlled only by multiple genetic factors. Both types of pattern show variations that may be correlated to a greater or lesser extent with environment, age and sex.

The pigmentation of *Xiphophorus maculatus* has been described in detail by Gordon (1931a), and much of what he recorded also holds for the other members of the genus. Rosen (1960) has described the outstanding pigmentary features of all the species and subspecies, and a more detailed account of the forms under current discussion may be found in Atz (1959a). In *X. maculatus,* the patterns of macromelanophores are governed by major genes that are dominant and sex-linked, while the major genes that influence the polymorphic micromelanophore patterns are dominant and autosomal. Although the distinction between the two types of melanophores is definite, genetically speaking, no diagnostic morphological features have ever been described. Macromelanophores are considerably larger than micromelanophores and may attain a diameter of 500 microns (Gordon, 1959), but the two types overlap in size. In practice, however, it is usually easy to distinguish between them because of the greater size and denser pigmentation of the macromelanophores.

1. Inheritance of Monomorphic Pigmentary Patterns in Hybrids.

That these pigment patterns are monomorphic precludes the use of most intraspecific crosses to analyze their genetic basis. Only mutants such as i for albinism or st for xanthism are available for genetic analysis. Interspecific or intraspecific hybridization provides, however, another means of revealing the hereditary foundation of species- or subspecies-specific characteristics. The individual genetic factors can seldom be identified, but the behavior of phenotypic characters or character-complexes may be studied, and from this conclusions may be drawn as to the nature of the genetic elements at work.

The many crosses between species and subspecies of *Xiphophorus* that were available made this type of analysis feasible. A series of pairs of opposing categories was set up (Tables II, III and IV) that represented pigmentary characters in which the parental species differed sufficiently to make the assignment of a hybrid to one or the other category, or to an "intermediate" one, not too arbitrary a procedure. Most of these fishes are partially covered by a pigmentary pattern that consists of parallel lines enclosing rhombic or hexagonal areas of lighter pigmentation. Superficially, this pattern appears to outline each scale, but its anatomical basis is formed by the scale pockets, each edge of which is bordered by a band of micromelanophores. This network has been appropriately called the *reticulum* by Rosen (1960), and it provides a natural basis for describing the patterns on the body since most of them, *e.g.* the *mid-lateral stripe,* can be considered as modifications of the reticulation (see Figs. 13, 18). *Vertical barring,* however, is clearly separable from the reticulum, lying deeper in the skin, as was pointed out by Gordon (1931a).[5] *Background pigmentation* and *inter-radial pigmentation* of the caudal fin are distinctive in *X. p. pygmaeus,* in which scale melanophores and skin

[5] Gordon considered this pattern to consist of *parr marks,* but any resemblance or relationship it may have to the well-known salmonid pattern is problematical. For one thing, unlike the latter, it is frequently retained as the fish matures. See Fig. 18.

TABLE II. SUMMARY OF "DOMINANCE" RELATIONSHIPS AMONG NON-POLYMORPHIC PIGMENTARY PATTERNS IN F_1 HYBRID *Xiphophorus*

	Total No. of Broods	Number of Broods:[1]		
		Intermediate	Resembling Female Parent	Resembling Male Parent
Reticulum				
Type	22	9	5	9
Extent	18	13	4	1
Mid-lateral Stripe	24	12	12	5
Background Pigmentation	4	0	1	3
Vertical Barring	22	18	5	3
Dorsal Fin Pattern	15	10	2	4
Caudal Fin Patterns				
Inter-radial Pigmentation	4	4	0	0
Caudal Edging	3	2	1	0
Ventral Edging	11	6	6	3
Dorsal Edging of Sword	3	1	0	3
Anal Fin, Caudal Edging	12	6	3	5
Mid-ventral Stripe	14	10	2	4
Deep-lying Spots	10	0	8	2
Totals	162	91	49	42

[1] Excess (20) above Total Number of Broods results from some broods having intermediate individuals in addition to those resembling either male or female parent, and therefore being recorded in two columns.

melanophores (neither of which is associated with the reticulum and thus may be considered to belong to the background) are by far the most poorly developed, and in which pigmentation is lacking between the finrays of the tail. A thin, dark border along the caudal edge of the caudal fin is found in some male *X. p. pygmaeus*, and a somewhat similar pattern along the caudal edge of the anal fin in female *X. maculatus*. In the swordtails, there is a dark band of pigmentation that runs along the caudal peduncle as the *mid-ventral stripe* which may or may not extend onto the ventral edge of the caudal fin. The dorsal edge of the sword of male swordtails may also be edged in black, and the way in which this is accomplished differs in different species. *Deep-lying spots* are a unique pattern of *X. couchianus* and consist of groups of deep-lying pigment cells, apparently associated closely with blood vessels, some of which are nevertheless visible along the posterior flanks of the fish.

The following observations have been made from a study of the data from Atz (1959a), summarized in Tables II, III and IV:

(1) The F_1s are not always uniform, either within broods or among similar crosses—even when judged by the relatively coarse standards employed.

(2) Among the F_1s, about as many characters resemble those of either parental form as are intermediate.

(3) Although characters in certain F_1s may resemble the female parent, there is, in the aggregate, no sign of maternal influence, since F_1 characters as frequently resemble the male parent.

(4) The F_2s are more variable than the F_1s, but not so in the expression of all, or even a majority of the characters.

(5) Backcrossing tends to result in characters that resemble the parental species with which the hybrid was backcrossed, but this is not invariably the case.

(6) Nevertheless, the second backcross never fails to produce at least some individuals that resemble the backcross species in the character in question, and usually the ma-

TABLE III. COMPARISON OF VARIABILITY IN NON-POLYMORPHIC PIGMENTARY PATTERNS BETWEEN F_2 AND F_1 HYBRID *Xiphophorus*

	Number of F_2 Broods Exhibiting:		
	No Increase in Variability	Increase Toward One Parental Form	Increase Toward Both Parental Forms
Reticulation			
Type	2	6	0
Extent	2	5	1
Mid-lateral Stripe	1	8	0
Background Pigmentation	1	1	0
Vertical Barring	3	5	0
Dorsal Fin Pattern	1	2	1
Caudal Fin Patterns			
Inter-radial Pigmentation	0	1	1
Caudal Edging	0	0	1
Ventral Edging	2	0	0
Dorsal Edging of Sword	2	0	0
Anal Fin, Caudal Edging	0	5	0
Mid-ventral Stripe	2	1	1
Deep-lying Spots	5	2	0
Totals	21	36	5

jority of the fish are practically indistinguishable from that species.

(7) A few characters, most notably ventral edging of the caudal fin, persist in some individuals after two backcrosses to the opposite parental species.

(8) Reciprocal crosses do not always produce similar offspring (*X. v. xiphidium* and *X. maculatus; X. m. cortezi* and *X. maculatus; X. h. guentheri* and *X. maculatus*).

(9) It seems impossible to predict, on any morphological basis, whether the expression of a given character will be "dominant," "recessive," or intermediate in the F_1.

(10) A character is occasionally "dominant" in one interspecific combination and "recessive" in another.

(11) Occasionally the expression of a character in the F_1 is noticeably different from that in either parent, sometimes resembling that of another species (Vertical bars like *X. v. xiphidium* in *X. couchianus* × *X. maculatus* and in *X. couchianus* × *X. v. variatus;* mid-lateral stripe like *X. v. variatus* in *X. h. strigatus* × *X. couchianus*).

2. Inheritance of Polymorphic Pigmentary Patterns in Hybrids.

The polymorphic pigmentary patterns of *Xiphophorus maculatus* have been extensively studied by Gordon and his collaborators, who have described them (Gordon, 1931a, 1948, 1951b; Gordon & Fraser, 1931), determined their mode of inheritance (Gordon, 1931b, 1937, 1947a, 1948, 1950a, 1956b), recorded and analyzed their frequencies in nature (Gordon, 1947a; Gordon & Gordon, 1957), and studied their development and physiology both in health and disease (Gordon, 1948, 1950a, 1951a,b, 1957, 1958a, 1959; Gordon & Smith, 1938; Nigrelli, Jakowska & Gordon, 1951). Similar, but not as extensive, studies have been made with five other species belonging to the genus *Xiphophorus*. Four of these are polymorphic, but only one, *X. variatus,* approaches the remarkable diversity of pigment patterns shown by *X. maculatus.*

a. Micromelanophore Polymorphic Patterns.

Many specimens of *X. maculatus, X. v. variatus* and *X. v. xiphidium* exhibit a distinctive arrangement of micromelanophores located on either side of the caudal peduncle at the base of the caudal fin and extending onto it in varying degrees. There are seven basic patterns in *X. maculatus* and four in *X. variatus,* some of which closely resemble each other. No fish ever carries more than two patterns, and genetic experiments

TABLE IV. EFFECTS OF BACKCROSSING ON NON-POLYMORPHIC PIGMENTARY PATTERNS OF HYBRID *Xiphophorus*

	First Backcross			Second Backcross			Third Backcross	
	Total Number Broods	Broods Showing Change Toward B. C. Parent	Broods Resembling B. C. Parent[1]	Total Number Broods	Broods Showing Change Toward B. C. Parent	Broods Resembling B. C. Parent[1]	Total Number Broods	Broods Resembling B. C. Parent
Reticulation								
Type	12	8	10	10	1	10	2	2
Extent	10	9	7(3)	6	1	6		
Mid-lateral Stripe	12	10	9(2)	10	3	8(2)	2	2
Background Pigmentation	1	1						
Vertical Barring	7	4	3(2)	4	2	2(2)	2	2
Dorsal Fin Pattern	7	6	5(1)	7	1	7	2	2
Caudal Fin Patterns								
Inter-radial Pigmentation	1	1	1					
Caudal Edging	1	1	1					
Ventral Edging	6	6	2(4)	7	2	4(3)	2	2
Dorsal Edging of Sword	1			4	1	4	2	2
Anal Fin, Caudal Edging	7	5	1(5)	5	1	3(2)		
Mid-ventral Stripe	7	6	(5)	9	1	4(4)	2	2
Deep-lying Spots[2]	3	3	(3)					

[1] Numbers in parentheses represent broods in which not all individuals resemble the backcross parent, and these are not included in the other figures in the same column. The number of broods in this column often exceeds the number of broods that show a change toward the backcross parent, because some F_1 hybrids already resemble the backcross parent in certain characters and many first backcross fish do.

[2] In one second backcross to *X. couchianus (X. maculatus* × *X. couchianus),* no deep-lying spots appeared, even though some of the first backcross fish had exhibited them.

have shown that each pattern is controlled by a member of a series of dominant, autosomal multiple alleles. Hybridization strongly indicates that the two series of alleles occupy the same locus in both species (Atz, 1959a). Two dominant, autosomal gene modifers that alter the appearance of the tail patterns in *X. maculatus* have been identified. One called *extensor* (E), changes the *comet* (Co) pattern, in which micromelanophores form a thin, dark border along the dorsal and ventral edges of the caudal fin, into the *wagtail* complex, in which all the fins and certain other extremities are pigmented (Gordon, 1946). The other modifier (Cg) changes the *twin-spot* pattern (T) into a reversed, C-shaped pattern called the *Guatemala crescent* (Gordon, 1956). Gordon found that these modifier genes are present in at least some *X. hellerii,* and he indicated that this species was perhaps their only source, the factors having entered the genome of various domesticated strains of *X. maculatus* by introgressive hybridization.

Evidence for the presence of an *extensor*-like gene in *X. v. xiphidium* was obtained in a brief series of crosses (Table V). A female *X. maculatus,* carrying the *comet* (Co) tail pattern, was mated to a male *X. v. xiphidium,* both parents being descended from wild-caught fish, and 51 of the 99 offspring showed *comet.* Of these, 14 had this pattern modified in the direction of the *wagtail* with an intensification and slight spread of the pattern itself, a darkening of part of each of the dorsal finrays and the appearance of heavy pigmentation on the upper and lower lips, the latter being a typical part of the *wagtail* complex (Fig. 1). When one of the hybrid females with this modified pattern was backcrossed to *X. v. xiphidium,* 30 of the offspring showed *comet* and

Table V. Influence of Hybridization on Micromelanophore Tail Patterns of Fishes of the Genus *Xiphophorus*

Factor	Parent with Pattern	Parent without Pattern	Modification of Pattern in Offspring	Cross[1]
Co	*maculatus* ♀	*xiphidium* ♂	Extension (slight *wagtail* effect) (see Fig. 1)	h20
Co	h20 ♀	*xiphidium* ♂	Enhanced *wagtail* effect (See Fig. 2)	h30
Co	h20 ♂	*xiphidium* × *variatus* ♀	Extension (slight *wagtail* effect)	h38
T	*maculatus* ♀	*guentheri* ♂	Extension (*Guatemala crescent*)	324
T	*maculatus* ♂	*cortezi* ♀	Extension (pseudo-crescent)	103B
O	*maculatus* ♀	*cortezi* ♂	Enhancement	80
Ct	*xiphidium* ♀	*variatus* ♂	Extension (pseudo-crescent) (see Fig. 7)	h4

[1] Number of cross, as designated in the records of the Genetics Laboratory of the New York Aquarium.

the 7 largest of these (at the time they were sacrificed) showed a more strongly expressed *wagtail,* with the caudal and dorsal finrays darkened for most of their length, the pectoral and anal finrays darkened to a lesser extent, and the lips heavily pigmented (Fig. 2). In contrast, when a hybrid male, showing a slightly developed *wagtail* pattern, was mated to a female intraspecific hybrid *X. v. xiphidium* × *X. v. variatus,* 57 of the offspring showed *comet* of which the 8 largest (at the time they were sacrificed) exhibited a *wagtail* pattern, better expressed than it had been among the members of the original hybrid cross, but not as strongly as it was among the backcross offspring just described. Gordon (1946) indicated that the *wagtail* pattern (CoE) was not apparent in young fish, but developed as they grew. This, however, cannot account for the absence of the *wagtail* effect in many of the Co fish, since some of these were adults when sacrificed and preserved. That the effect was enhanced by backcrossing to *X. v. xiphidium* indicates that more than one modifying gene was involved.

In a cross between a *twin-spot X. maculatus* and a swordtail from British Honduras, *X. hellerii guentheri,* all the hybrids that inherited T exhibited the *Guatemala crescent.* This pattern was compared, partly by means of cleared specimens, with some of the *Guatemala crescent* fish studied by Gordon (1956b). As far as the tail pattern was concerned, the fish appeared identical. In addition to the tail pattern, however, there is a modification of pigmentation near the mouth of *Guatemala crescent* fish (Gordon, 1956b). Most prominent are two spots of heavy pigmentation at each mandibular junction. These were lacking in the present specimens; instead they showed a crescent of dense pigmentation immediately behind the upper lip, overlying the ethmoid region posteriorly and the heads of the premaxillae and maxillae anteriorly. The pigment was located in the dermis. An examination of *Guatemala crescent* fish in the collection of the Genetics Laboratory (which could not include all the specimens or crosses studied by Gordon) revealed that in only one other cross (250) did the TCg fish exhibit the crescent-shaped pigmented area behind the upper lip. The ancestry of the latter cross involved *X. maculatus* from both the Rio Jamapa and domestic sources and *X. hellerii strigatus* from the Rio Papaloapon. It thus revealed no obvious genetic relationship to the present cross.

Another modification of the *twin-spot* pattern occurred in hybrids of *X. m. cortezi* and *X. maculatus,* in which a shadowy, crescentic pattern was formed by micromelanophores more or less connecting the upper and lower spots. In the hybrids from another cross involving the same species, the *one-spot* tail pattern was larger than it ever appears in the parental species, *X. maculatus.*

The offspring of an intraspecific cross, involving a female *X. v. xiphidium,* and a male *X. v. variatus,* exhibited a complete range of tail pat-

terns from *cut-crescent* to *crescent*. (Fig. 7), although the *cut-crescent* of the female parent was entirely normal in appearance, and no *crescent*-bearing fish is known among the ancestors of the fish.

None of the offspring from the above crosses was bred, and so no information exists on the genetic behavior of their modified tail patterns; nor is there any other cross involving a fish carrying genes for the same tail patterns, which might indicate how widespread the supposed genetic modifiers may be.

b. Macromelanophore Polymorphic Patterns.

The patterns composed of macromelanophores are all polymorphic, that is, none of them occurs in every individual of the species. They can most simply be described as *spotted,* and the spots may be considered to vary from the size of single macromelanophores to broad bands of black pigment. Because of the relatively large size of macromelanophores and the concentration of melanin granules within them, these patterns appear darker and more sharply demarcated from their background than do the patterns composed of micromelanophores. Variation in location, size and number of spots is therefore readily apparent, and this circumstance may contribute significantly to the impression that the variability of the macromelanophore patterns is considerably greater than the variability of the micromelanophore ones. There is, however, no question about the greater range of expressivity of the major genes controlling macromelanophores in hybrid genomes, in which this may be increased to pathological melanosis on the one hand or reduced to no penetrance at all on the other (Gordon, 1951a).

Five basic macromelanophore patterns found in *X. maculatus* have been described by Gordon (1951b, pp. 175-179) and Gordon & Gordon (1957, pp. 3-6):

Spotted (Sp)—irregular spotting on sides.

Nigra (N)—irregular blotches or bands on sides.

Striped (Sr)—discrete rows of spots on sides.

Spotted dorsal (Sd) — irregular spotting on dorsal fin.

Spotted belly (Sb)—heavy spotting on belly and ventral and anal fins.

With rare exceptions, no more than two of these patterns occur in a fish, and there is good evidence that they are controlled by dominant, sex-linked alleles (Gordon, 1948). Crossing-over has occurred, however, so that two macromelanophore genes have become located on a single chromosome (MacIntyre, 1961). The series might therefore better be designated as pseudoallelic.[6]

Gordon (1943) recognized a single macromelanophore pattern in *X. variatus* and in *X. xiphidium* (at that time considered to be separate species rather than subspecies as they are today), *viz., spotted* (Sp) but subsequent, unpublished, analysis revealed the presence of a second pattern, *spotted caudal.* Rosen (1960, p. 81), however, lists four macromelanophore patterns from *X. variatus, viz., spotted* (which he calls *blotched*), *spotted caudal, speckled* and *black-banded.* The latter three occur only in fish from the Rio Cazones, from which no living specimens have yet been collected for genetic studies. The *spotted* (Sp) pattern is inherited as a sex-linked dominant and, according to Atz (1959a), appears to be an allele of the Sp of *X. maculatus.*[7]

The *spotted* pattern in *X. variatus xiphidium* typically consists of numerous, somewhat diffuse spots located in the region of the mid-lateral line and immediately above it.[8] In size they approach, but never equal, the area of the exposed portion of a scale, but the great majority are considerably smaller, that is, less than half as large. As few as eight spots have been found on one side of a fish, but the number is usually very much more. A common variant of this pattern (in certain populations) is one in which the spots are so numerous that they form, at a distance, a band of pigmentation along the side of the fish. Closer examination reveals that this is composed of numerous closely grouped macromelanophores, many of them "touching" one another. Aside from a tendency to follow the reticular pattern (see below) and be concentrated near the mid-lateral line, however, no pattern can be discerned. Sometimes the distribution of macromelanophores is so generalized that the whole body of the fish above the region of the mid-lateral line appears flecked with pigment. In such fish, individual spots are hard to distinguish.

The majority of the larger spots in *X. v. xiphidium* are roundish, but the smaller ones take on less regular, more elongate forms. They usually appear more diffuse and less clearly defined than do the spots found on *X. montezumae cortezi,*

[6] It was evidence and considerations of this nature that led Rosen (1960, pp. 76-77) to state that the macromelanophore genes "are not all members of a single allelic series."

[7] See Figs. 4-6 which show the P_1 and some of the F_1s of one of the crosses that revealed this relationship.

[8] See the P_1 male in Fig. 10 as an example of the appearance of Sp in this subspecies.

X. hellerii and, in some instances, *X. maculatus*. This may be the result of either or both of two factors: (1) as far as can be determined, the number of pigment cells per unit area of a spot is definitely less in *X. v. xiphidium* than in either *X. hellerii* or *X. montezumae*, and (2) there may be a lower concentration of pigment per cell in *X. v. xiphidium*. This could account for the differences observed between it and some strains of *X. maculatus*, where the number of cells appears to be substantially the same. A third possibility, of course, is that the macromelanophores are of different sizes, but those from the two swordtails appeared smaller, if anything, than those in *X. v. xiphidium*. This possibility could only be settled by treating live specimens with adrenaline to concentrate as uniformly as possible the pigment in the melanophores, fixing the fish in that state, and then making counts and measurements.

Although the *spotted* pattern of *X. v. xiphidium* is characterized by its variability and lack of definition, one feature always marks its distribution. This is its close spatial relationship to the reticulum; it is very rare that a spot or individual macromelanophore is found that does not seem to be touching or lying astride the reticulum — or is not occupying a place that would have shown reticulum, had it been present. In those cases, described above, where macromelanophores are so numerous that they practically form a horizontal band near the mid-lateral line, numerous cells must fall in areas between reticular elements, but this occurs only where the cells are so numerous that they "touch" or "overlap" one another. Even in these cases, the cells at the edges of the pigmented areas follow the reticulum.

The *spotted* (Sp) pattern of *X. variatus variatus* resembles that seen in *X. v. xiphidium* but differs in the following ways.[9] Typically the pattern is confined to the region of the mid-lateral line, but the spots are not concentrated in the latter region as frequently as they are in *X. v. xiphidium*. There are more large spots and no fish without large spots, except those from certain regions. There are numerous roundish spots, but because the spots sometimes follow the reticulum very closely, V- and Y-shaped ones are not rare. In some specimens from one region (Rio Tempoal), the spots so closely follow the reticulum that a pattern strongly reminiscent of the *striped* (Sr) pattern of *X. maculatus* is produced. Although the number of pigment cells per unit area within a spot appears comparable to that in *X. v. xiphidium*, the intensity and sharpness of the spots is in general greater than in the latter subspecies. The range of variability of the *spotted* pattern is, however, greater in *X. v. variatus*.

Two macromelanophore patterns, *spotted* (Sp) and *spotted caudal* (Sc), are known in *X. montezumae cortezi* and each appears to be controlled by a single dominant, autosomal gene (Atz, 1959a). These are not alleles nor is Sc an allele of the Sp factor of *X. hellerii guentheri*, but no direct evidence exists concerning their relationship to the macromelanophore factors in other species. In *X. m. cortezi*, the *spotted* (Sp) pattern typically consists of prominent, deeply pigmented, roundish spots mostly confined to the mid- and post-dorsal regions, above the mid-lateral stripe, and to the dorsal and caudal fins. In size, individual spots approach, but rarely if ever exceed, the area of the exposed portion of a scale. Although there may be as few as five spots on one side of an adult-sized fish, there are usually many more. Analysis indicates that the number of spots increases with size, and presumably age, and that males exhibit more spots than females (Atz, 1959a). On the average, the closer one approaches the mid-dorsal line, the greater the density of spots (Fig. 13). The macromelanophores that comprise this pattern appear to be associated in some way with the reticulum, which is especially well developed in this species (Fig. 13). No spot was ever found that was not "touching" some part of the reticular pattern, that is, no spot was located entirely within the hexagonal or trapezoidal areas formed by the reticular elements. Usually the spots appeared to be directly astride the reticular bars and sometimes a halo effect was noticeable. In connection with the latter phenomenon, it should be noted that macromelanophores are located at or very near the same level in the dermis as are the micromelanophores. Another characteristic of the spots of *X. m. cortezi* is that they not infrequently coalesce, forming irregularly shaped blotches. On the dorsal fin, the spots are found in the interradial membranes, although they may extend over the finrays. In heavily spotted males, they tend to form two rows, one near the base of the fin, the other about halfway between base and distal edge. The spots on the dorsal fin are usually larger than those on the body. In females or immature fish, the *spotted* pattern rarely extends onto the dorsal fin. In some *spotted* males and a few females, there may be spots on the caudal fin as well. These are spindle-shaped or oval and generally small. They lie between or along the caudal finrays and have been seen to occur

[9] See the *spotted* hybrids in Figs. 8 and 9 for unmodified examples of this pattern. Fig. 18 shows an enlargement of some macromelanophore spots from this subspecies.

between any two finrays except those involved in the sword. They seldom occur under the superficial caudal musculature at the base of the fin, but rather in the middle half of the exposed portion of the fin itself.

The tendency for macromelanophores to congregate in the caudal fin of *X. m. cortezi* is most strikingly seen in the *spotted caudal* (Sc) pattern. This consists of one or more irregular, elongate patches of heavy pigmentation, commencing close to the base of the caudal finrays and extending toward the rear for roughly one-third of the fin's length and ending in a variable number of irregular, tapering extensions of pigmentation (Fig. 15). These extensions usually are apposed to a finray; in fact, the whole pattern appears to develop in close relation to the caudal finrays. The macromelanophores invade the perimysia of the superficial muscle of the caudal fin as well as enveloping the finrays. In adult fish, the pattern may be only a sliver of pigmentation or it may be a blotch covering the second through the twelfth caudal finrays. Such large blotches are rare, however. Although the most frequent number of pigmentary patches comprising this pattern is one, two are not uncommon, and as many as four may be seen.

Macromelanophore spotting is known to occur in a small proportion of the individuals of two subspecies of *X. hellerii* (Rosen, 1960, pp. 120 and 126). In the form presently available, *X. h. guentheri,* genetic data indicate that the *spotted* (Sp) pattern is inherited as an autosomal dominant. Aberrant ratios have been noted, however, which might be explained by incomplete penetrance of Sp. It is noteworthy that true-breeding spotted *X. h. guentheri* were established in our Genetics Laboratory only after individuals exhibiting spots had been selected regularly to carry on the line for more than seven generations (Kallman, personal communication). Crosses with *X. maculatus* bearing Sr or Sd showed that these two factors are not alleles of the Sp from *X. h. guentheri* (Gordon, 1958b; Atz, 1959a). This pattern is notable for its relatively small number of large, intensely dark, irregularly shaped spots (Fig. 19). The irregularity is partly, but not wholly, the result of the coalescence of adjacent spots as a result of increase in size. Because of the discreteness of the spots, it is easy to study their morphology individually, and a series of stages beginning with a single macromelanophore and extending through intermediate stages (of, say, 30 macromelanophores) to large spots composed of uncountable numbers of pigment cells (in the order of hundreds but probably less than one thousand cells) could be distinguished. The most simple explanation is that the spots increase in size through the appearance of more pigment cells. There is good evidence that the number of spots increases with the size of fish and therefore presumably with age (Atz, 1959a). The usual maximum size of a single spot, *i.e.,* one not involved in any coalescence with other spots, approaches the area of the exposed portion of a scale. Although the poor development of the reticulum makes the determination of relationship difficult, in all instances where such a determination could be made, the spots were seen to be closely associated with reticular elements. Frequently a halo effect is in evidence. The location of the spots on the body follows no discernible pattern save that they are much more frequently found on or above the mid-lateral stripe and somewhat more often in the pre- and mid-dorsal regions.

The effects of hybridization on macromelanophore patterns in 108 crosses are outlined in Table VI. The changes in the phenotypic expression of eight major genes belonging to five different species and subspecies vary from no discernible effect to loss of penetrance, on the one hand, and severe melanosis with the production of melanotic overgrowths, on the other. These results may be summarized by stating that they confirm and extend the conclusions reached by Gordon (1951b). The following are the observations resulting from an analysis of Table VI:

(1) Genes vary in their ability to respond to genetic influence from foreign genotypes. The gene for *spotting* (Sp) in *X. maculatus* is unquestionably the most potent in this respect; in no other species has it failed to produce well developed melanosis and, at least occasionally, neoplastic overgrowths in the form of melanomas (see items nos. 1-21 of Table VI). At the other extreme stand the genes for spotting in *X. hellerii guentheri* and *X. montezumae cortezi* whose expressivity changes to only a limited extent (nos. 82-91, 117-143) except in one hybrid combination (nos. 92-95).

(2) Species vary in their tendency to influence foreign genes controlling pigmentation. In *X. maculatus,* the species whose pigmentary genes are most capable of showing excessive growth in foreign genotypes, no enhancement of pigmentation belonging to patterns from other species has ever occurred (nos. 66-68, 78-80, 84, 98, 117-140). If any species could be assigned the role of being most likely to show melanosis and melanoma as a result of the introduction of foreign pigmentary genes into its genotype, *X. hellerii* would be the choice, based on its reactions to Sp, Sb, Sd and N (Gordon,

TABLE VI. INFLUENCE OF HYBRIDIZATION ON MACROMELANOPHORE PATTERNS OF FISHES OF THE GENUS *Xiphophorus*

No.	Parent with Pattern	Parent without Pattern	Offspring with Pattern[1]	Cross[2]
		Sp — X. maculatus		
1	*maculatus* ♂	*couchianus* ♀	F_1: Melanosis	h7
2	h7	h7	F_2: Severe melanosis to enhancement	h7[2]
3	*maculatus* ♀	*couchianus* ♂	F_1: Melanosis	h15
4	h15 ♀	*couchianus* ♂	BC: Melanosis (5) overgrowths (1)	91
5	h15 ♂♂♂	*couchianus* ♀♀♀	BC: Melanosis (4), severe melanosis (7), overgrowths (2)	92, 94, 99
6	99 ♂	*couchianus* ♀	2nd BC: Melanosis (6 out of 8)	100
7	91 ♀	*couchianus* ♂	2nd BC: Melanosis	101
8	*maculatus* ♀	*couchianus* ♂	F_1: Melanosis	845
9	845 ♀	845 ♂	F_2: Severe melanosis (2 out of 13) to moderate enhancement (6 out of 13); overgrowths (1 out of 13)	878
10	845 ♂	845 ♀	F_2: Severe mleanosis (2 out of 11) to moderate enhancement (2 out of 11)	880
11	845 ♀	*couchianus* ♂	BC: Severe melanosis	881
12	881 ♀	*couchianus* ♂	2nd BC: Severe melanosis	945
13	881 ♀	*couchianus* ♂	2nd BC: Severe melanosis	946
14	*maculatus* ♀	*couchianus* ♂	F_1: Severe melanosis	851
15	851 ♂	851 ♀	F_2: Severe melanosis (8) to melanosis (2)	893
16	851 ♂	*couchianus* ♀	BC: Severe melanosis (2); overgrowth (1)	934
17	*maculatus* × *variatus* ♂	*variatus* ♀	BC: Melanosis (3 out of 33); enhancement (see Figs. 4-6)	h61
18	*maculatus* ♂	*cortezi* ♀	F_1: Strong enhancement (9); melanosis (4); overgrowths (7)	103
19	*maculatus* ♂	*cortezi* ♀	F_1: Strong enhancement	103B
20	103	*cortezi*	BC: Melanosis (5); overgrowths (5)	103BC
21	h7 ♀	h3 ♂	Enhancement	h29
		Sd — X. maculatus		
22	*maculatus* ♂	*couchianus* ♀	F_1: Reduced penetrance	h7
23	*maculatus* ♂	*couchianus* ♀	F_1: Mild melanosis	325
24	*maculatus* ♀	*couchianus* ♂	F_1: No penetrance (14)	845
25	*maculatus* ♀	*couchianus* ♂	F_1: Melanosis	895
26	*maculatus* ♀	*xiphidium* ♂	F_1:Reduced penetrance (see Fig. 1)	h20
27	*maculatus* ♂	*xiphidium* ♀	F_1: No penetrance (see Fig. 3)	h31
28	*maculatus* ♀	320 ♂	BC: Mild melanosis; overgrowth (1)	350
29	*maculatus* × *strigatus* ♂	*guentheri* ♀	BC: Melanosis; overgrowths (2 out of 8)[3]	479
30	479 ♂	*guentheri* ♀	2nd BC: Severe melanosis (2); melanosis (6); enhancement (1); melanomas (4)[3]	671
31	479 ♀	*strigatus* ♂	2nd BC: Severe melanosis (1); melanosis (3); melanomas (2)[3]	672
32	h7 ♂	h2 ♀	No penetrance	h28
		Sr — X. maculatus		
33	*maculatus* ♂	*couchianus* ♀	F_1: No effect	68
34	68	68	F_2. Reduced penetrance and expressivity	68[2]

TABLE VI. INFLUENCE OF HYBRIDIZATION ON MACROMELANOPHORE PATTERNS OF FISHES OF THE GENUS *Xiphophorus* (*Continued*)

No.	Parent with Pattern	Parent without Pattern	Offspring with Pattern[1]	Cross[2]
35	*maculatus* ♀	*couchianus* ♂	F_1: Enhancement	895
36	895 × 895		F_2: Mild melanosis (1), enhancement (6), reduced expressivity (3), strongly reduced expressivity (4)	895[2]
37	*maculatus* ♂	*xiphidium* ♀	F_1: Slight enhancement (see Fig. 3)	h31
38	*maculatus* ♂	h2 ♀	Reduced expressivity	h22
39	*maculatus* ♀	*cortezi* ♂	F_1: Slight suppression	h80
40	*maculatus* ♂	320 ♀	BC: Enhancement to nearly complete suppression (see Fig. 21)	384
41	*maculatus* ♀ × 384 ♂		2nd BC: Enhancement to nearly complete suppression	419
42	384 ♀	*maculatus* ♂	2nd BC: Reduced expressivity	486
43	486 ♀ × 486 ♂		No effect	523
44	486 ♀ × 486 ♂		No effect	524
45	486 ♀	486 ♂	No effect	525
46	*maculatus* ♂	320 ♀	BC: Slight enhancement	385
47	385 ♀	*maculatus* ♂	2nd BC: Reduced expressivity	421
48	421 ♂	*guentheri* ♀	Enhancement	458
49	458 ♀ ×458 ♂		Enhancement to nearly complete suppression	521
50	*maculatus* ♀	320 ♂	BC: Enhancement	386
51	*maculatus* ♀ × 386 ♂		2nd BC: Reduced expressivity	420
52	420 ♀ × 420 ♂		No effect to nearly complete suppression (see Fig. 22)	522
		Sp — X. variatus variatus		
53	*variatus* ♂	*couchianus* ♀	F_1: No effect (see Fig. 8)	h23
54	*variatus* ♀	*couchianus* ♂	F_1: No effect	h13
55	h13 ♂	h13 ♀	F_2: No effect	h13[2]
56	*maculatus* × *variatus* ♂	*variatus* ♀	BC: No effect (see Figs. 4-6)	h61
57	*variatus* ♂	*cortezi* ♀	F_1: No effect (see Fig. 9)	h8
58	*variatus* ♀	*pygmaeus* ♂	F_1: Enhancement (1 out of 27) (see Fig. 11)	h1
59	*variatus* ♂	*pygmaeus* ♀	F_1: No effect	h11
60	h11	h11	F_2: No effect	h11[2]
61	*variatus* ♀	*xiphidium* ♂	F_1: No effect	h2
62	h2	h2	F_2: No effect	h2[2]
63	*variatus* ♂	*xiphidium* ♀	F_1: No effect	h3
64	h3	h3	F_2: No effect	h3[2]
65	*variatus* ♂	*xiphidium* ♀	F_1: No effect (see Fig. 7)	h4
66	h2 ♀	*maculatus* ♂	No effect	h22
67	h4 ♀	h20 ♂	No effect	h38
68	h2 ♀	h7 ♂	No effect	h28
		Sp — X. variatus xiphidium		
69	*xiphidium* ♂	*cortezi* ♀	F_1: Strong enhancement (see Fig. 10)	903
70	*xiphidium* ♂	*cortezi* ♀	F_1: Strong enhancement	914
71	*cortezi* × (*cortezi* × *xiphidium*) ♂[4]	*cortezi* ♀	2nd BC: Severe melanosis with overgrowth (3)	941

TABLE VI. INFLUENCE OF HYBRIDIZATION ON MACROMELANOPHORE PATTERNS OF FISHES OF THE GENUS *Xiphophorus* (*Continued*)

No.	Parent with Pattern	Parent without Pattern	Offspring with Pattern[1]	Cross[2]
72	*xiphidium* ♂	*pygmaeus* ♀	F_1:Mild melanosis (27 out of 28) (see Fig. 12)	h66
73	h66 ♀	*pygmaeus* ♂	BC: Enhanced melanosis (3 out of 9); reduced melanosis (2 out of 9)	106
74	*xiphidium* ♂	*evelynae* ♀	F_1: No effect	913
75	*xiphidium* ♂	*variatus* ♀	F_1 No effect	h2
76	h2	h2	F_2: No effect	h2[2]
77	*xiphidium* ♀	*variatus* ♂	F_1: Enhancement (see Fig. 7)	h4
78	*xiphidium* ♀	*maculatus* ♂	F_1: No effect (see Fig. 3)	h31
79	*xiphidium* ♂	*maculatus* ♀	F_1: Slight suppression to no effect (see Fig. 1)	h20
80	h20 ♀ × *xiphidium* ♂		F_1 & BC: No effect (see Fig. 2)	h30
81	h2 ♀	h7 ♂	Slight suppression	h28
		Sp — X. montezumae cortezi		
82	*cortezi* ♀	*couchianus* ♂	F_1: Reduced expressivity	849
83	*cortezi* ♀	*cortezi* × (*cortezi* × *xiphidium*) ♂[4]	2nd BC: Slightly reduced expressivity	941
84	*cortezi* ♂	*maculatus* ♀	F_1: No effect	h80
85	*cortezi* ♂	*pygmaeus* ♀	F_1: No effect	h12
86	h12	h12	F_2: No effect	h12[2]
87	*cortezi* ♀	*strigatus* ♂	F_1: No effect	h10
88	*cortezi* ♀	*strigatus* ♂	F_1:Reduced expressivity and penetrance (see Fig. 13)	h9
89	h9 ♂	*strigatus* ♀	BC: Slight suppression (see Fig. 14)	h27
90	h27 ♀ × h27 ♂		No enhancement	h27[2]
91	h27[2] ♀	*strigatus* ♂	Slight suppression	h39
92	*cortezi* ♂	*montezumae* ♀	F_1: No effect	900a
93	*cortezi* ♂	*montezumae* ♀	F_1: Enhancement (8 out of 11 ♂♂, 1 out of 12 ♀♀)	900b
94	*cortezi* ♂	*montezumae* ♀	F_1: No effect	900c
95	*cortezi* ♂	*montezumae* ♀	F_1: Strong enhancement (3 out of 9 ♀♀), enhancement (1 out of 9 ♀♀) (no Sp ♂♂ present)	900d
		Sc — X. montezumae cortezi		
96	*cortezi* ♀	*couchianus* ♂	F_1: No penetrance (3 adult, 16 immature)	849
97	*cortezi* ♀	*variatus* ♂	F_1: No penetrance (see Fig. 9)	h8
98	*cortezi* ♂	*maculatus* ♀	F_1: No penetrance	h80
99	*cortezi* ♀	*strigatus* ♂	F_1: Enhancement (see Fig. 15)	h26
100	h26 ♀	*cortezi* ♂	BC: Enhancement (2); no enhancement (1)	h40
101	h26 ♀	*strigatus* ♂	BC: Melanosis (1); enhancement (greater than in h40) (17)	h41
102	h41 ♀, h41 ♂	*strigatus* ♂, ♀	2nd BC: Enhancement (3); no enhancement (4)	h44, h45
103	h41 ♀	*strigatus* ♂	2nd BC: Severe melanosis (3); overgrowth (1); enhancement (16) (see Fig. 16)[3]	h42
104	h42	h42	Severe melanosis (2); overgrowths (5); melanosis to enhancement (31)	h42[2]

TABLE VI. INFLUENCE OF HYBRIDIZATION ON MACROMELANOPHORE PATTERNS OF FISHES OF THE GENUS *Xiphophorus* (*Continued*)

No.	Parent with Pattern	Parent without Pattern	Offspring with Pattern[1]	Cross[2]
105	h42 ♀	*hellerii* (albino) ♂	3rd BC: Mild melanosis (3); overgrowths (2); enhancement (6)	h47
106	h42 ♀, h42 ♂	*strigatus* ♂♀	3rd BC: Severe melanosis (3); overgrowth (1); mild melanosis (2); enhancement (10); no effect (2)	h50
107	h50 ♀ × h50 ♂		Severe melanosis (2); overgrowths (5); enhancement (5); no enhancement (5)	353
108	h41 ♀ × h41 ♂		Enhancement	h43
109	h43 ♀ × h43 ♂		Melanosis (5); overgrowths (2;) enhancement (5)	h46
110	h46	h46	Severe melanosis (1); melanosis (5); overgrowth (1); enhancement (2)	h46[2]
111	h46[2]	h46[2]	Severe melanosis (1); enhancement (6); no enhancement (3)	h46[3]
112	h42[2] ♀	*hellerii* (*wagtail*) ♂	Overgrowth (1 out of 3)	314
113	*cortezi* ♂	*montezumae* ♀	F_1: Enhancement (6 out of 9 ♂♂, 1 out of 4 ♀♀)	900a
114	*cortezi* ♂	*montezumae* ♀	F_1: Enhancement (3 out of 11 ♂♂, 1 out of 8 ♀♀)	900b
115	*cortezi* ♂	*montezumae* ♀	F_1: Enhancement (3 out of 5 ♂♂, 2 out of 3 ♀♀), melanosis (2 out of 5 ♂♂)	900c
116	*cortezi* ♂	*montezumae* ♀	F_1: Strong enhancement (3 out of 11 ♀♀), enhancement (7 out of 11 ♀♀) (1 Sc ♂ present)	900d
		Sp — X. hellerii guentheri		
117	*strigatus* × *guentheri* ♂	*maculatus* ♀	F_1: No effect	322
118	*guentheri* ♂	*maculatus* ♀	F_1: No effect (1)	324
119	*guentheri* ♀	*maculatus* ♂	F_1: No effect (see Fig. 20)	320
120	320	320	F_2: No effect	320[2]
121	320[2]	320[2]	F_3: Slight suppression	320[3]
122	320[3]	320[3]	F_4: Slight enhancement	320[4]
123	320 ♂	*maculatus* ♀	BC: Reduced penetrance and expressivity	350
124	320 ♂	*maculatus* ♀	BC: No effect	386
125	386 ♂	*maculatus* ♀	2nd BC: No effect	420
126	420 ♀	420 ♂	Slight enhancement (3 out of 35) (see Fig. 22)	522
127	320 ♀	*maculatus* ♂	BC: No effect (see Fig. 21)	384
128	384 ♂	*maculatus* ♀	2nd BC: Slight suppression	419
129	384 ♀	*maculatus* ♂	2nd BC: No effect	486
130	486 ♀	486 ♂	No effect	523
131	486 ♂	486 ♀	No effect	524
132	486 ♀ × 486 ♂		No effect	525
133	320 ♀	*maculatus* ♂	BC: No effect	385
134	385 ♀	*maculatus* ♂	2nd BC: Reduced expressivity	421
135	421 ♀	*guentheri* ♂	No effect	485
136	421 ♂	*guentheri* ♀	No effect	458

TABLE VI. INFLUENCE OF HYBRIDIZATION ON MACROMELANOPHORE PATTERNS OF FISHES OF THE GENUS *Xiphophorus* (*Continued*)

No.	Parent with Pattern	Parent without Pattern	Offspring with Pattern[1]	Cross[2]
137	458 ♀	458 ♂	No effect	521
138	*guentheri* ♀	*maculatus* × *strigatus* ♂	BC: Slight enhancement (1 out of 8) [5]	479
139	479 ♂	*guentheri* ♀	2nd BC: No effect [5]	671
140	479 ♀	*strigatus* ♂	2nd BC: Slight suppression [5]	672
141	*guentheri* ♂	*strigatus* ♀	No effect	321
142	*guentheri* × (B.C. and inbred, ScCoE, *hellerii-cortezi* hybrid ♂	*strigatus* ♀	No effect	751
143	751 ♂	*strigatus* ♀	BC: No effect	887

[1] Described by means of a somewhat arbitrary series of classes, ranging from complete absence of macromelanophore pattern to melanoma, all but the first two categories being concerned with expressivity:

No penetrance
Reduced penetrance
Nearly complete suppression
Reduced expressivity
Slight suppression
No effect (*i.e.*, normal)
Slight enhancement
Enhancement
Strong enhancement
Mild melanosis
Melanosis
Severe melanosis
Overgrowths (melanoma)

Numbers in parentheses indicate the number of fish

[2] Number of cross as designated in the records of the Genetics Laboratory of the New York Aquarium. When more than one macromelanophore pattern was involved, the cross has been listed under each genetic factor.

[3] Photographs of other melanotic members of h42 may be found in Gordon (1951b, p. 199) and as Fig. 1 of Marcus & Gordon (Zoologica, 39: 123-131, 1954). The caption of the latter erroneously implies that the fish belong to h50.

[4] Fish received in 1958 from Dr. Curt Kosswig (see Kosswig (1959) for a general account of the genetic background of this fish).

[5] In designating backcrosses (BC), *X. hellerii strigatus* and *X. hellerii guentheri* have been equated.

1948, and nos. 28-31) and Sc (nos. 99, 101-112).

(3) The same gene may vary both positively and negatively under the influence of different genomes. Sd may show reduced penetrance or none at all (Gordon, 1951a and nos. 22, 24, 26, 27, 32) or melanosis with melanoma (Gordon, 1951a and nos. 23, 25, 28-31). Similarly Sr exhibits both reduced and enhanced expressivity (Gordon, 1948 and compare nos. 33-34, 38, 39 with 35, 36, 37), while Sc varies in expression from no penetrance to severe melanosis and melanoma (compare nos. 96-98 with 99-112).

(4) Crosses involving the same pigment patterns and species sometimes give different results (compare nos. 1, 3 and 8 with 14; 40 with 46 and 50; 41 with 42; 87 with 88; 92 and 94 with 93 and 95; 102 with 103; 123 with 124, 127 and 133). This probably indicates that several genes and their alleles are involved in the modification of the pigmentary patterns and that different combinations of these are present in different individuals.[10]

(5) Backcrossing to the parental form from which the macromelanophore pattern originated lessens the enhancing or reducing effect of hybridization on its expression (compare nos. 40 with 42, 46 with 47, 50 with 51, 99 with 100 and 101). Gordon & Smith (1938) backcrossed a melanotic F_1 hybrid, *X. maculatus* × *X. variatus xiphidium*, to *X. maculatus* and obtained fish

[10] The age at which the fish were sacrificed is a variable that was controlled only in a general way, and this undoubtedly is the reason for some of the varied results. In the examples given above, however, the broods were of roughly the same age or the differences were so striking that ontogenetic stage could not account for them. At any rate, these crosses serve as a salutary warning against dogmatic assertions unless they are backed up by a replication involving several of the same crosses made with fish from different genetic strains.

with no melanosis, and Kosswig (1948) reported results of a similar nature.

(6) Backcrossing to the parental form that did not carry the macromelanophore pattern increases the enhancing or reducing effect of hybridization on its expression (compare nos. 4 and 5 with 3, 11 with 8, 73 with 72, 101 with 99). A plateau is soon reached, however, and further backcrossing may even result in a diminution of the effect (compare nos. 6 with 5, 7 with 4, 12 and 13 with 11, 20 with 18, 102 with 101, 105 and 106 with 102 and 103).

(7) Inbreeding frequently increases the range of phenotypic expression of macromelanophore patterns (compare 2 with 1, 9 and 10 with 8, 34 with 33, 36 with 35, 49 with 48). Two series of successive inbreedings showed variability in the average expression of the pigmentary pattern, the mean or mode of expression not necessarily being the same for successive generations (nos. 108-111 and 120-122). Gordon & Smith (1938) also obtained more variable F_2 offspring in two crosses of *X. maculatus* × *X. couchianus* and one of *X. maculatus* × *X. variatus xiphidium*.

(8) The same individuals may show enhancement of one macromelanophore pattern and no change or reduction in the expressivity of another (compare nos. 28 and 123, 30 and 139, 31 and 140, 26 and 79, 40 and 127, 48 and 136, 49 and 137, 50 and 124, 57 and 97, 71 and 83, 92 and 113, 94 and 115).[11]

IV. Discussion

1. Genetics of Micromelanophore Pigmentary Patterns as Revealed by their Appearance in Hybrids.

The behavior of the reticulum and other nonpolymorphic micromelanophore patterns in interspecific crosses within the genus *Xiphophorus* strongly indicates that their inheritance is controlled by polygenes, that is, by relatively numerous factors, each of which has a small phenotypic effect. This is in accord with the conclusions reached by Kosswig (1948) and with analyses indicating the type and number of genes involved in the species differences of other kinds of animals (Dobzhansky, 1937). The intermediate appearance of the F_1, the more variable F_2, and the return in appearance toward successive backcross parents are all classical indications of polygenic inheritance. In none of these ways, however, did the present hybrids perform entirely in the classical manner; in fact, unorthodox responses were sometimes as much in evidence as classical ones. For example, approximately the same number of characters in the F_1 resemble either parent as are intermediate (see Table II and discussion on p. 176). Although the classical genetic behavior of the present hybrids would be hard to explain except on the basis of polygenes, the failure to conform to the above criteria does not indicate the converse. The appearance of unstable developmental systems as a result of the mixing of two foreign, mutually unadapted genotypes (Schmalhausen, 1949; Lerner, 1954) could very well bring about seemingly inconsistent results. Such a reduction in genetic homeostasis, as Lerner has designated it, could also account for the striking lack of uniformity occasionally seen in F_1 broods in which these hybrids were as variable as the F_2 broods arising from them, or even more so. Gordon & Rosen (1951) also found high variability in two gonopodial characters of F_1 hybrids between *X. maculatus* and *X. hellerii*. It is of interest to note that Hubbs & Strawn (1957) have warned that high variability in a population of fish is not a safe criterion for hybrid fertility, that is, for the presence of F_2, F_3 . . . F_n generations, because high variability is sometimes exhibited by the F_1—which may be sterile. Hubbs (1956) attributed this variability to the combination of dissimilar developmental rates of the parental forms, and this may be considered a special case of the more general phenomenon of genetic homeostasis mentioned above.

Genetic modification of the polymorphic tail patterns has been an accepted but meagerly documented part of the concept of hereditary influences on pigmentation in fishes of the genus *Xiphophorus*. Hybridization has served to reveal additional examples of this phenomenon. Although the presence or absence of each polymorphic pigmentary pattern is typically controlled by a single gene, numerous other genes undoubtedly influence its phenotypic expression. The problem of identifying genic modifiers, the vast majority of which have small *plus* or *minus* effects, is at present insurmountable. The specific effects of two modifications of the tail patterns of *X. maculatus* have, however, been described by Gordon and attributed to two dominant, nonallelic genes. Similar modifications have appeared in the tail patterns of a few of the present interspecific hybrids (Table V).

On one occasion the *wagtail* effect was observed when a female *X. maculatus* carrying the

[11] Gordon (1958b) briefly reported on the first three crosses listed here.

comet (Co) pattern was crossed with *X. v. xiphidium*. Gordon (1946) showed that this *extensor* effect, in which supplementary pigmentation appears around almost all of the finrays, the mouth and the operculi, results from the presence of a single dominant autosomal gene (E) that does not belong to the series of multiple alleles affecting tail patterns of which *comet* is a member. He also showed that this factor was present in *X. hellerii*. Whether the same factor is present in *X. v. xiphidium* is problematical, however, since the *wagtail* pattern is much more variably and also less clearly expressed in the *maculatus–xiphidium* hybrids than in the interspecific crosses in which Gordon was able to demonstrate the presence of E. Moreover, backcrossing to *X. v. xiphidium* heightened the expressivity of the *wagtail* pattern, an effect not seen in Gordon's fish, since the pattern appeared in the F_1 full-blown, so to speak. At the very least, however, we may conclude that in some individuals of *X. v. xiphidium*, there are genes capable of modifying the *comet* pattern of *X. maculatus*.

Gordon (1956b) described the modification of the *twin-spot* (T) pattern of *X. maculatus* to form the *Guatemala crescent*, and he presented genetic evidence that a dominant, autosomal, independently segregating gene is responsible, which he designated Cg. He indicated that this gene was probably present in an appreciable number of *X. hellerii*. This pattern appeared, but in a slightly modified form, when a *twin-spot X. maculatus* was crossed with *X. hellerii guentheri*. This modification may be the result of additional modifying factors or may represent the phenotypic expression of an allele of Cg. Another modification of the *twin-spot* pattern of *X. maculatus* appeared in a cross with *X. montezumae cortezi*. The extension of pigment was, however, less definite than in the *Guatemala crescent* and was not associated with any extra pigmentation around the mouth. With regard to other possible modifications of tail patterns in hybrids involving *X. montezumae cortezi*, it might be mentioned that in a cross with a *one-spot* (O) *X. maculatus*, this pattern was exceedingly intense. The puzzling relationship of *crescent* (C) to *cut-crescent* (Ct) shown in one cross between two subspecies of *X. variatus* could only be elucidated by further crosses. It does serve to emphasize, however, the similarity of the developmental processes involved in the two patterns.

2. Genetics of Melanosis and Melanoma in Hybrids.

The striking exaggeration of expression that certain macromelanophore patterns exhibit under the influence of foreign genotypes has attracted more attention than any other feature of the genetics of *Xiphophorus*. Gordon (1951b, 1957) has reviewed the extensive experiments performed with *X. maculatus*, *X. hellerii* and their hybrids and summarized the concepts that have been developed from them. Crosses involving these and other members of the genus in new combinations have yielded results that are most easily interpreted in the same way. They therefore add to the confidence with which we may accept the concepts developed by Gordon over the years.

a. Capacity for Atypical Growth and Specificity of Macromelanophore Genes.

Gordon (1948) pointed out that the different macromelanophore patterns of *X. maculatus* are enhanced to different degrees in hybrids with *X. hellerii*, and Gordon & Smith (1938) and Gordon (1951b) showed that the same pattern, *viz.* the *spotted* pattern of *X. maculatus*, is enhanced to different degrees in hybrids with different species. The same relationships hold for the macromelanophore patterns of species other than *X. maculatus* (Table VII). On the basis of present work and that of Gordon (1948, 1951b), the allelic series of sex-linked dominants may be arranged according to their potency for melanosis and melanoma production in hybrid combinations:

Sp (spotted) *X. maculatus*
Sb (spotted belly) *X. maculatus*
N (nigra or black-sided) *X. maculatus*
Sd (spotted dorsal) *X. maculatus*
Sr (striped) *X. maculatus*
Sp (spotted) *X. variatus xiphidium*
Sp (spotted) *X. variatus variatus*

The specificity that these genes exhibit in their morphological manifestations must, of course, be the product of equally specific physiological processes, and these genetically controlled events maintain a definite measure of specificity under the abnormal conditions imposed by hybridization, even though the limits of their phenotypic variability may be considerably increased. It is to be noted, for example, that certain hybrids in which two major macromelanophore genes are present show one pigmentary pattern in an enhanced form while the other remains within normal limits or even suffers some loss in expressivity (Table VI, and item 8 on p. 169). One of the reasons for the series of backcrosses and inbreedings conducted with the offspring of the cross of a spotted *X. hellerii guentheri* with *X. maculatus* (Table VI, nos. 119, 133-137) was

TABLE VII. MACROMELANOPHORE GENES OF FISHES OF THE GENUS *Xiphophorus* RANKED ACCORDING TO THEIR CAPACITY FOR ATYPICAL GROWTH IN HYBRIDS[1]

Factor	Species	Location of Spotting	Capacity for Atypical Growth	Mode of Inheritance	Relation to Other Genes
Spotted (Sp)	*X. maculatus*	Body and tail	Melanomas in some F_1 combinations and all backcross combinations[2]	Sex-linked dominant	Allelic
Spotted Dorsal (Sd)	*X. maculatus*	Dorsal fin	Melanomas in some F_1 and backcross combinations[2]	Sex-linked dominant	Allelic
Striped (Sr)	*X. maculatus*	Body and tail	Melanomas in one backcross combination[2]	Sex-linked dominant	Allelic
Spotted (Sp)	*X. variatus xiphidium*	Body and tail	Melanomas in one 2nd backcross combination; mild melanosis in two F_1 combinations[2]	Sex-linked dominant	Allelic
Spotted Caudal (Sc)	*X. montezumae cortezi*	Caudal fin	Melanomas in one 2nd backcross combination; strong enhancement in two F_1 combinations[2]	Autosomal dominant	Non-allelic[4]
Spotted (Sp)	*X. montezumae cortezi*	Body, tail, dorsal and caudal fins	Strong enhancement in one F_1 combination	Autosomal dominant	Non-allelic[4]
Spotted (Sp)	*X. variatus variatus*	Body and tail	Enhancement in one F_1 combination[3]	Sex-linked dominant	Allelic
Spotted (Sp)	*X. hellerii guentheri*	Body and tail	None	Autosomal dominant	Non-allelic[4]

[1] Based on Gordon (1948, 1951b) and data from Table VI.

[2] Backcross combinations refer only to backcrosses to the species or subspecies from which the macromelanophore pattern did not originate.

[3] Enhancement exhibited by a single fish out of a total of more than 200 hybrids. Kosswig (1937) states that the dominant, sex-linked gene P of *X. v. variatus* (probably identical with Sp) is responsible for spots that are more weakly expressed in hybrids with *X. hellerii* than they are in the parental species, but more strongly than they are in hybrids with *X. maculatus*. As far as crosses with *X. hellerii* were concerned, this observation was based on tri-hybrids produced by Kosswig (1935a, b) in which *X. maculatus*–*X. hellerii* hybrids were crossed with *X. v. variatus*. Rust (1941) notes that the spots of P are formed by macromelanophores. His illustration of a spotted hybrid from the cross, *X. hellerii* ♀ × *X. v. variatus* (P) ♂ shows no enhancement of this pattern. Rust also describes the inheritance of a dominant, sex-linked gene O of *X. v. variatus*, which is expressed as black spots on a light orange background. According to Kosswig (1948), this factor gives rise to tumors when introduced into the *hellerii* genome. Rust does not mention any pigmentary abnormalities in his paper, but he illustrates two O-bearing fish, the offspring of second backcrosses to *X. hellerii*, that show signs of a dark, pigmented overgrowth in the region near the base of the sword. Unfortunately, all fish carrying the O gene were lost during World War II (Kosswig, *in litt.*).

[4] None of these genetic factors appears to be allelic; as far as known they occupy non-homologous chromosomes.

to test what might for convenience be called the integrity of the Sp gene. The rationale was to introduce the gene into the *X. maculatus* genotype by the appropriate cross, followed by several backcrosses to *X. maculatus*. Reintroducing the gene into *X. h. guentheri* would reveal a modification of it (through the acquisition of gene modifiers or some change in the gene itself) in the form of enhanced expressivity. Inbreeding at various stages in the process would serve to increase the variability of genetic combinations and bring out latent factor interaction. The anticipated negative results were obtained in full measure; no change could be detected.

On the other hand, the fact that *X. maculatus*, the species in which the most potent melanosis- and melanoma-producing genes occur, has never been known to give rise to hybrids exhibiting any enhancement of patterns from other species (Table VI, nos. 79, 84, 98, 119) points to some similarity in physiological process underlying the actions of all the macromelanophore genes (Atz, 1959b). According to Gordon's hypothesis of the integration of macromelanophore genes into the genotype of *X. maculatus* (see p. 173), this species possesses several modifying genes that control the growth of its own macromelanophores. Presumably this control is general enough to exercise restraint on macromelanophore genes from other species, and this in turn presupposes some similarity in the biochemical processes of the genes being regulated. The existence of species like *X. hellerii*, the hybrids of which seem to be especially likely to exhibit enhanced pigmentary patterns, similarity provides support for the idea of biochemical characteristics common to all macromelanophore genes; but the fact that the hybrids of *X. couchianus* and *X. p. pygmaeus*, both of which lack macromelanophore patterns, do not show as great a degree of atypical macromelanophore growth as do those of *X. hellerii* and *X. m. cortezi*, which do have such patterns, indicates important differences between the macromelanophores of *X. maculatus* and *X. variatus* on the one hand and those of *X. hellerii* and *X. m. cortezi* on the other. Evidently the ability of the latter two species to control the growth of their own macromelanophore patterns confers little or no ability to control the growth of others. Of less critical value is the observation that the manifestations of atypical growth shown by the macromelanophores from different species appear to be basically the same. In all known hybrid combinations, melanosis necessarily precedes melanoma. Reed, Gordon & Lansing (1933) and Gordon & Smith (1938) have described several characteristics in which the melanoses and melanomas of *maculatus-couchianus* hybrids resemble those of the more completely described *maculatus-hellerii* ones, and Gordon & Nigrelli (1949) stated that the melanomas found in *cortezi-hellerii* hybrids are histologically not unlike those found in other combinations.

Anders *et al.* (1961, 1962) have reported that the amount of free amino acids in various species of *Xiphophorus* varies inversely with the ability of their macromelanophores to produce tumors in hybrids: *X. maculatus* has the smallest quantity, *X. variatus xiphidium* a greater amount, *X. montezumae cortezi* still more and *X. hellerii guentheri* the greatest. This is the order in which the authors rank the species according to the potency of their macromelanophores for atypical growth in various hybrid combinations, *X. maculatus* having the most potent genetic factors and *X. hellerii* the least. This arrangement agrees substantially with the one in Table VII, which is based on independently acquired data. The significanee of the apparent relationship between tumor production or susceptibility and amino acids has yet to be explained.

b. Evidence for the Evolution of Macromelanophore Genes and Their Polygenic Modifiers.

The tendency for increased variability in the F_2, the return toward the parental type in one backcross and the exaggeration of the modifying effect in the other are all most easily accounted for by assuming the presence of several modifying factors, as Kosswig (1931) did in order to explain similar findings involving *X. hellerii* and *X. maculatus*. Kosswig and Gordon (1937) explained their results with these two species on the basis of a few, perhaps only two, pairs of modifiers. The similarity of results between the present crosses and theirs, particularly the rapidity with which a plateau of enhancement is reached with repeated backcrosses to the P_1 carrying the modifying genes, indicates that approximately the same number of modifiers is involved in the presently studied species interactions.

Gordon (1950b, 1951b, 1958a), and Gordon & Gordon (1957) considered the production of melanosis and melanoma in *Xiphophorus* hybrids from an evolutionary point of view. They described how the combination of a potentially harmful gene, like Sp, and a series of modifying genes that render it harmless might have arisen. When the mutation to Sp first occurred, it is supposed to have been detrimental in a manner similar to the way Sp leads to melanosis and melanoma when introduced into foreign gen-

omes today.[12] Fisher (1928) and others have shown that such a gene, whose over-all effect is deleterious, will accumulate genic modifiers that tend to make the expression of its harmful effects recessive and, sometimes, eventually mask them completely—providing that the locus in question continues to yield mutant genes of the type upon which natural selection can operate. If this has indeed been the history of the integration of macromelanophore genes into the genotype of *X. maculatus*, the genotypes of the other species belonging to the genus *Xiphophorus* might better be considered to lack the genes or alleles that control the expression of the macromelanophore gene, rather than to possess genes that enhance its expression, as postulated by Kosswig(1931), or to remove a specific inhibitor that controls its normal growth and multiplication, as suggested by Gordon & Smith (1938). This clarifying view, which represents more than just a change in the terminology used to describe the interacting genomes, was suggested in 1958 by Donn E. Rosen, who was then a member of the staff of the Genetics Laboratory of the New York Aquarium.

Gordon (1951a, b, 1957) showed that the same kinds of abnormalities in the development of pigment patterns occur in intraspecific hybrids as do in interspecific ones, although they are not as strongly expressed. He considered this to be good evidence that subspecies and other geographically isolated populations of *Xiphophorus* are proceeding along paths of genetic differentiation similar to those already taken by the species. In a small series of crosses between the two subspecies of *X. montezumae*, two macromelanophore patterns from one of them were enhanced to an abnormal degree (Table VI, nos. 93, 95, 113-116). This provides another example of incipient melanosis, presumably the result of the mixing of different constellations of modifying genes that have evolved at the subspecific level of genetic differentiation.

In evaluating the significance of the pigment cell abnormalities of hybrids as a criterion of taxonomic relationship, Rosen (1960) pointed out that the degree of abnormality could act as a measure of the degree of genetic difference and should, therefore, enter into the determination of systematic relationship. He indicated, however, that the correlation between the relative taxonomic position (as determined by a whole array of pertinent data) of any two non-interbreeding groups (species, subspecies or merely river-system populations) and the hybrid pigmentary abnormality exhibited by their hybrids is far from perfect. The present data support this view. For example, intraspecific crosses, between subspecies, may or may not result in offspring with atypical macromelanophore patterns (Table VI, nos. 61-65, 74, 75-77, 92-95, 113-116, 141). In interspecific crosses, the same macromelanophore gene may lead to an enhanced pattern in some combinations or a normal pattern, or none at all (zero penetrance) in others (Table VI, compare 69, 70, 71 with 78 and 79; 99 with 97, 98). Moreover, in the same interspecific cross, one pattern may be enhanced and another unaffected or reduced in expressivity (Table VI, compare 18 and 19 with 98; 99 with 87 and 88). It is nevertheless true that pigmentary abnormalities in interspecific hybrids are more severe and more frequently encountered than in intraspecific ones, as Gordon (1951b) and Rosen (1960) have indicated. Yet even here the distinction is not absolute, for one case is known in which the hybridization of subspecies produced a more atypical pattern than did crosses with four other species (Table VI, compare 93, 95 with 82, 84-88; 113-116 with 96-98).

In his illuminating discussion of homology, de Beer (1958) cited a case of pigment pattern modification in a moth that closely resembles the situation in two geographically isolated populations of *Xiphophorus maculatus* described by Gordon (1951a). When a platyfish from the Rio Coatzacoalcos that carries the *spotted dorsal* gene (Sd) is crossed with a platyfish from the Rio Jamapa, the expressivity of the gene is enhanced, the pattern in the hybrid covering a considerably greater area of dorsal fin and adjacent body than the *spotted dorsal* of either pure race, both of which are very similar in appearance. Similarly, when Ford (1953) crossed individuals belonging to a dark subspecies of *Triphaena comes* from two widely separated islands, the expression of the pattern was modified. Since other tests had shown that the same principal gene was responsible for the color pattern on both islands, the change in its expressivity in the hybrid must have resulted from the interaction of two different systems of genetic modifiers, each of which had nevertheless been responsible for producing an identical phenotype. De Beer (1958) concluded that although the two pigment patterns were pheno-

[12] A difficulty of this hypothesis is that it makes no provision for the development of the multiple allelism often associated with polymorphism. It therefore implies that despite the apparent similarity of the polymorphism of *X. maculatus* and *X. variatus* to that found in other kinds of animals (most notably insects), its origin has not been the same (see, for example, Ford, 1957).

typically alike and were controlled by the same principal gene, they were not homologous since they were the result of parallel evolution from a common ancestor that undoubtedly did not exhibit the dark pattern, at least not in the form in which it exists at present. The same conclusions could be reached with regard to the situation Gordon found in *X. maculatus.*

A somewhat different situation has been found in two of the subspecies of *X. variatus.* Each of these has at least one *spotted* (Sp) macromelanophore pattern, and the two studied at this time are morphologically distinguishable (see p. 162). In only one of a number of crosses between *X. v. variatus* and *X. v. xiphidium* did any sign of atypical expression of their spotted patterns occur (Table VI, compare 77 with 61-65, 75, 76). Evidently these two alleles are similar enough to be controlled by the same set or sets of modifying factors, and evidently the constellation of modifying genes is the same or nearly the same in both subspecies. Nevertheless, when associated with hybrid genomes involving two other species, *X. p. pygmaeus* and *X. montezumae cortezi,* these alleles react quite differently (Table VI, compare 58-60 with 72, 73; 57 with 69-71). Presumably the relatively slight difference between them could become manifest in a genetic environment where the major gene and approximately half of its polygenic modifiers were not mutually adapted and therefore allowed less "margin for error" during ontogenesis, that is, maintained a lower level of genetic homeostasis.

c. Atypical Pigmentation Associated with the Sc Gene.

Kosswig (1936) was the first to describe the enhancement of the *spotted caudal* pattern (Sc) of *X. montezumae cortezi* in hybrids with *X. hellerii.* In a preliminary report, Gordon (1947b) pointed out that melanomas were obtained "not in the first generation hybrids, but in some of the backcrosses of the hybrids to *X. hellerii* and in some of the second inbred generation." According to the Laboratory data gathered together in Table VI, melanotic overgrowths were obtained among the second backcross hybrids and in the second inbred generation of the first backcross hybrids (nos. 103 and 109). The specimens available at the present time show the following general relationships between type of cross and severity of pigmentary abnormality:

F_1—enhancement

1st backcross—melanosis

Inbreeding, 1st generation—enhancement

Inbreeding, 2nd generation—melanoma

Inbreeding, 3rd generation—melanoma

Inbreeding, 4th generation—melanosis

2nd backcross—melanoma

Inbreeding—melanoma

3rd backcross—melanoma

Inbreeding—melanoma

The percentage of melanomatous individuals in any one brood varied from, roughly, 5% to 30%. The variability of the extent to which melanoses and melanomas were exhibited by various backcross broods may indicate that the factors favoring enhancement of pigment cell growth are not uniformly distributed in *X. hellerii.* There is, however, a complicating factor that makes the results appear distinctly less uniform than those from comparable crosses between *X. hellerii* and macromelanophore-carrying *X. maculatus,* namely, the slow development of melanosis and the late appearance of melanoma among these fish. For example, in our present strain of melanomatous *hellerii-cortezi* hybrids (RJ), which is maintained by repeated backcrossing to *X. hellerii,* overgrowths do not appear until the fish are nearly two years old (Klaus D. Kallman, personal communication). Unless all specimens are maintained for uniformly long periods of time, which was not the case with those discussed above, an uncontrolled variable of major proportions will have been introduced.

Gordon & Nigrelli (1949) briefly described the histology of the overgrowths associated with the Sc pattern. They found that these melanomas somewhat resemble the others that occur in the tail region of various hybrids—presumably associated with Sp, Sb and Sd of *X. maculatus* since these factors also lead to overgrowths on the caudal peduncle. As in all of the melanomas, melanosis always precedes the appearance of any Sc overgrowth. This sequence has been witnessed several times, and individuals with overgrowths always had well developed melanoses while the reverse was frequently not the case. The extensive invasion of tissues by black pigmentation begins near the juncture of caudal fin and peduncle, that is, the general location of the Sc pattern. The gradual encroachment of the melanosis up the caudal peduncle and, subsequently, the body has been observed, but all excessive pigmentation does not arise from this single spreading concentration. In many of the Sc hybrids, clusters of macromelanophores were seen in areas quite far anterior to the melanotic region on the peduncle (Figs. 16, 17). Slashes of intensely black pigmentation also sometimes appeared in the dorsal fin and, less frequently, the pectorals. The latter, it should be

noted, are never pigmented in normal fish of either of the parental species. Whether these islands of macromelanophores hasten the forward advance of melanosis or contribute materially to its development cannot be determined from preserved material, but it seems likely. In general, the number of clusters of macromelanophores could be roughly correlated with the extent of pigmentation of the hybrid brood in question, but this was not always the case. For example, clusters of macromelanophores were seen in seven fish that showed no sign of the Sc pattern (4 from no. 107, 1 from 109 and 2 from 112). The smallest specimen that exhibited macromelanophore clusters was 9 mm. in standard length.

The cases of extreme melanosis, involving at least half of the body in addition to the tail, and those of melanotic overgrowths, were not distributed equally between the sexes of Sc hybrids:

No. males with extreme melanosis	6
No. males with melanoma	0
No. females with extreme melanosis as well as melanoma	3
No. females with extreme melanosis but without melanoma	1
No. females with melanoma but without extreme melanosis	11

The melanoses of the males tended to be greater, even when compared with the most extremely melanotic females. One second backcross male (h42) was completely melanotic except for the eyes and tip of the gonopodium. No connection between sex and melanosis or melanoma has ever been established in the hybrids of *X. hellerii-X. maculatus,* and Berg & Gordon (1953) found melanomas in five hybrids that had no detectable gonads. Nevertheless, the possibility that sex hormones or some genetic factor associated with sex may influence melanoma development in hybrids among the species of *Xiphophorus* may again be broached, on the basis of present observations.

Gordon (1956a) presented a brief, popular account of the origin of the Red Jet (RJ) strain in which he called attention to two noteworthy genetic interactions responsible for the striking red and black coloration of these fish. The strain originated with the crossing of a *wagtail* swordtail with an Sc-bearing member of the inbred offspring of a second backcross to *X. hellerii* of *hellerii-cortezi* hybrids (Table VI, no. 112). The *wagtail* fish must have acquired its pattern through hybridization with *X. maculatus* carrying the *comet* (Co) factor (Gordon, 1946), and therefore a modicum of genes from that species must be present in the Red Jet strain. Gordon (1956a) reported that in this strain, the macromelanophore *spotted caudal* (Sc) pattern is more enhanced when the micromelanophore *wagtail* (CoE) pattern is present.

This factor interaction is similar to the one between the micromelanophore *stippled* (St) and macromelanophore *spotted* (Sp) patterns in *X. maculatus* which Gordon (1928) also described. That this interaction takes place between cells or elements of tissue is indicated by the very localized enhancement of macromelanophore patterns that is occasionally seen at places on the bodies of hybrids where concentrations of micromelanophores occur. For example, an F_2 fish from the cross of a *striped, one-spot* (SrO) *X. maculatus* with *X. couchianus* (Table VI, no. 36) exhibited a large, melanotic spot on either side of the caudal peduncle, where the *one-spot* pattern is located. The Sr pattern in this fish was barely perceptible, that is, it showed reduced expressivity. At least one other sib, and perhaps three, showed an early stage of a similar macromelanophore spot. In the latter three fish, the Sr pattern was fairly well developed but not enhanced in any way—except in the immediate vicinity of the *one-spot.*[13]

Gordon (1956a) indicated that the red coloration of the Red Jet strain was the result of an enhancement of the three or four horizontal red stripes characteristic of the subspecies *X. hellerii guentheri.* Gordon (1948, 1950a) had described how the red-colored patterns of *X. maculatus* are enhanced in *hellerii-maculatus* hybrids and how erythrophoromas appear, in rare instances, among such hybrids. Similar interactions can occur in other kinds of hybrids, and we have seen *X. maculatus-X. couchianus* hybrids in which the *red dorsal* (Dr) pattern of the common platyfish parent was considerably enhanced. Kosswig (1937, 1948, 1959) has indicated that the same pattern is enhanced in hybrids with *X. v. variatus* and that similar intensification and spread of red pigment patterns occur in hybrids with *X. variatus xiphidium.*

3. Further Aspects of Hybridization.

In his critical review of what is known about hybridization among North American fishes, Hubbs (1955) states that "It has proved to be an almost universally valid rule that natural inter-

[13] Another indication of localized interaction between pigment cells is the halo effect in which a macromelanophore spot is surrounded by an area that appears to be free of all melanophores. (See Fig. 13.) Enzymatic competition for a limited amount of substrate might be the explanation, and a search for unpigmented pigment cells within the halo should be made. (See p. 162 for additional comment on the halo effect.)

specific hybrids are intermediate between their parental species in all characters in which those species differ, whether they be external or internal, of shape, color, form, structure, or numbers of parts (vertebrae, gillrakers, finrays, teeth)—except for some features that reflect hybrid vigor." There is no question that the overall appearance of the vast majority of fish hybrids is intermediate between the parental forms, but when a detailed, character-by-character comparison is made, an appreciable number of characters may be found to resemble one parent only. The present hybrids illustrate this very well (Table II), and numerous other examples have been reported in the literature (Atz, 1959a). Such instances of "dominance" do not, of course, indicate that the character in question is inherited in a simple Mendelian fashion, as Newman (1914) recognized. The genotypes of even closely related species differ in many genes (Dobzhansky, 1937), and polygenic inheritance is to be expected in the vast majority of cases. There may exist, however, some regular association between the relative amounts of "dominance" and intermediateness that are exhibited by an F_1 hybrid and the phylogenetic relationship of the parental forms. The more closely related the parents are, the less loci are involved in genetic differences, not only in the aggregate but presumably in the control of individual characters as well. The smaller the number of different alleles involved in determining a character, the greater the chance of those from one parent being completely dominant over those from the other. From this it may be deduced that, in general, the greater the proportion of F_1 hybrid characters resembling one of the parental forms rather than being intermediate, the closer to each other are the parents phylogenetically. This may be the reason why a relatively large proportion of characters in the present hybrids show "dominance" of one parental character or the other, while many of the hybrids described by Hubbs and others are intermediate to a significantly greater extent. Moreover, there are instances among the present hybrids in which a character unlike that in either parental form appeared, or in which a character resembling that found in a third species appeared (see item 11 on p. 158).

Results remarkably similar to the ones obtained with the present hybrids were described by Newman (1914) for the pigmentation of F_1 hybrids among *Fundulus heteroclitus, F. majalis* and *F. diaphanus*.[14]

Although the present specimens were not examined for signs of hybrid incompatibility other than pigmentary ones, this phenomenon was manifest in abnormal sex ratios and the presence of large, sexually undifferentiated fish. In general there was a shortage of males, which is in accord with Haldane's Rule that the heterogametic sex is more likely to be sterile, rare or absent in animal hybrids. As far as is known, the male *Xiphophorus* is heterogametic save for the platyfish that inhabit British Honduras (Gordon, 1957). These and other manifestations of hybrid incompatibility have been discussed in detail by Rosen (1960). We need only point out that in no species combination where a concerted effort has been made has it proved impossible to obtain F_1, F_2, F_3 or backcross generations.[15] Hybrid sterility may in fact exist between some species of *Xiphophorus,* since reduced fertility is frequently encountered, but this has not yet been demonstrated.

It must be clear from the preceding discussion (see especially pp. 172-173) that atypical pigment cell growth in *Xiphophorus* cannot be considered an isolating mechanism, as Crew (1940) and Stebbins (1958) have done. That some isolating mechanism must exist is evident, however, from the following observations: (1) *X. hellerii* and *X. maculatus* are found side-by-side in several river systems in southern Mexico, Guatemala and British Honduras; *X. hellerii* and *X. v. variatus* both inhabit at least one tributary of the Rio Nautla; *X. v. variatus, X. p. pygmaeus* and *X. montezumae cortezi* exist close to one another in the Rio Axtla and all three species have been caught there in a single pool (by Gordon, Atz and F. G. Wood, Jr. in 1948); (2) All of these particular species combinations have produced hybrids in the laboratory (Table I); (3) Among thousands of specimens of *Xiphophorus* collected in nature, not a single hybrid has ever been discovered (Rosen, 1960). Laboratory studies (Clark, Aronson & Gordon, 1954) and data collected in the field (Rosen, 1960) indicate strongly that a complex of factors, is responsible for keeping the sympatric species of *Xiphophorus* reproductively apart. The relative importance of the several factors and details of how they operate have yet to be determined.

V. Summary

1. Previous genetic studies of fishes of the genus *Xiphophorus* have concentrated on *X. maculatus, X. hellerii* and their hybrids. The

[14] This paper was not seen until after the present data had been gathered and analyzed.

[15] Tri-hybrids have been produced involving *X. maculatus, X. variatus* and *X. couchianus* (Table VI, nos. 21, 32, 68, 81) and *X. maculatus, X variatus* and *X. hellerii* (Kosswig, 1935a,b). The former were never bred, but Kosswig found that the latter were fertile.

present studies are principally concerned with five additional species and subspecies (*X. couchianus, X. variatus xiphidium, X. v. variatus, X. montezumae cortezi, X. pygmaeus pygmaeus*) and may be summarized by stating that they confirm and extend the work of Myron Gordon on this group of poeciliid fishes.

2. The pigmentary patterns of these fishes may be separated into monomorphic and polymorphic components and the latter into patterns composed of micromelanophores or macromelophores. The behavior of the monomorphic patterns in hybrid crosses shows that they are controlled by polygenes and that, in some cases the number of genes involved is not large.

3. The changed appearance of polymorphic pigmentary patterns in various hybrid combinations also reveals probable methods of genetic control; both micro- and macromelanophore patterns are undoubtedly influenced by constellations of modifying genes. A few new examples of modified tail patterns (micromelanophore) and several of spotted patterns (macromelanophore), including the *spotted caudal* pattern, have been studied. In hybrids, tail patterns resembling the *wagtail* and *Guatemala crescent* and spotted patterns ranging from zero penetrance to melanosis and melanoma production have been observed.

4. The major pigment cell genes vary in their ability to produce melanosis and melanoma in hybrids, and the species vary in their ability to control the growth of pigmentary patterns from other forms. *X. maculatus,* which possesses the genes most potent in producing atypical pigment cell growth in hybrids, is also the only species in whose hybrids no enhancement of macromelanophore patterns from other species ever occurs.

5. This indicates a similarity of biochemical processes among all the major genes for macromelanophore patterns, but a highly developed specificity also exists, because in the same hybrid individual, the expressivity of one macromelanophore pattern may be increased while that of another is reduced or remains unchanged.

6. The concept of genetic homostasis, as developed by Schmalhausen and by Lerner, has proved useful in explaining departures from the results expected in typical polygenic inheritance and also the differences in expressivity in hybrids of genetic factors that act practically the same when associated with genomes more like their normal ones.

7. The appearance, in various hybrids, of the *spotted* patterns of *X. variatus xiphidium* and *X. v. variatus,* and crosses between *X. montezumae cortezi* and *X. m. montezumae,* indicate that the same kinds of genetic differentiation occur in subspecies as in species.

8. The frequency with which the characters of hybrids resemble those of either parent, rather than being intermediate, may be a measure of the phylogenetic relationship of the parental forms. The more closely these are related, the greater the proportion of characters that are not intermediate.

9. No absolute hybrid sterility between any of the fishes of the genus *Xiphophorus* has ever been demonstrated. Although the abnormal expressivity of pigment genes in hybrids leads to lethal melanoma in some crosses, it seems never to have served as an isolating mechanism.

VI. References

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EXPLANATION OF THE PLATES

Living and preserved fishes photographed by Sam Dunton, Photographer, New York Zoological Society. Prepared skins photographed by Dr. Ross F. Nigrelli, Pathologist, New York Aquarium, New York Zoological Society.

Dimension of fishes is Standard Length and is approximate.

Plate I

Fig. 1. Hybrid *X. maculatus* × *X. variatus xiphidium.* Male on the right shows *wagtail*-like modification of *comet* (Co) pattern of *maculatus* and slightly suppressed *spotted* pattern of *variatus.* Both males show the *crescent* (C) tail pattern. See Table V and Table VI, nos. 26, 79.

Fig. 2. Backcross offspring, males (*X. maculatus* × *X. v. xiphidium*) × *X. v. xiphidium* Upper fish exhibits *wagtail*-like modification of *comet* (Co) pattern. See Table V and VI, no. 80.

Fig. 3. Hybrid *X. v. xiphidium* × *X. maculatus.* Female (32 mm.) on right, exhibits a slightly enhanced *striped* (Sr) pattern from *maculatus.* Male (30 mm.), on left, exhibits *spotted* (Sp) pattern from *xiphidium.* See Table VI, nos. 27, 37, 78.

Plate II

Fig. 4. *Xiphophorus v. variatus,* female (40 mm.), on left. Hybrid, *X. maculatus* × *X. v. variatus,* male (35 mm.), on right. Male exhibits *spotted* (Sp) pattern from *variatus,* the spots being small and located in front of and under the dorsal fin. Male also exhibits *spotted* (Sp) pattern from *maculatus,* the spots being large and located under the dorsal fin and behind it. (P_1 of h61).

Fig. 5. Backcross offspring, females (31-35 mm.), showing different degrees of enhancement of the *spotted* pattern from *maculatus.* (h61).

Fig. 6. Backcross offspring, males. Two upper fish exhibit the *spotted* pattern from *variatus*; lowest, exceptional fish the *spotted* pattern from *maculatus.* Three exceptional (crossover?) males and one female appeared among 41 females and 53 males. No fish exhibited both *spotted* patterns, thus, indicating that the two Sp factors are alleles. (h61). See Table VI, nos. 17, 56.

Plate III

Fig. 7. Hybrid *X. v. xiphidium* × *X. v. variatus.* Both fish exhibit an enhanced *spotted* (Sp) pattern from *xiphidium.* Female, on right, also exhibits the *spotted* (Sp) pattern from *variatus,* the spots of which are much smaller and lighter. Male, on left, exhibits a modified *cut-crescent* tail pattern; female (35 mm.) a typical *upper cut-crescent* pattern. See Table V, Table VI, nos. 65, 77.

Fig. 8. Hybrid *X. couchianus* × *X. v. variatus.* Female, above, exhibits *spotted* (Sp) pattern from *variatus.* See Table VI, no. 53.

Plate IV

Fig. 9. Hybrid *X. montezumae cortezi* × *X. v. variatus.* Female, on right, exhibits *spotted* (Sp) pattern from *variatus.* See Table VI, nos. 57, 97.

Fig. 10. *X. m. cortezi* × *X. v. xiphidium.* P_1 male (25 mm.), upper right, exhibits the *spotted* (Sp) pattern. P_1 female (35 mm.), upper left, exhibits no macromelanophore pattern. F_1, lower left, exhibits strongly enhanced *spotted* pattern from *xiphidium;* F_1 lower right, exhibits no macromelanophore pattern. See Table VI, no. 69.

Plate V

Fig. 11. Hybrid *X. v. variatus* × *X. p. pygmaeus.* Male (25 mm.), below, exhibits *spotted* (Sp) pattern from *variatus.* As this fish grew older, the spots increased several times in size and new ones appeared in abnormal locations. See Table VI, no. 58.

Fig. 12. Hybrid *X. p. pygmaeus* × *X. v. xiphidium.* Female, on left above, exhibits strongly enhanced *spotted* (Sp) pattern from *xiphidium.* See Table VI, no. 72.

Plate VI

Fig. 13. *X. m. cortezi* × *X. hellerii strigatus.* P_1 female, on left below, exhibits *spotted* (Sp) pattern. F_1 male, on right above, exhibits same *cortezi* pattern but with reduced expressivity. See Table VI, no. 88. Note prominent reticulum of *cortezi.* (h9).

Fig. 14. Backcross offspring of h9 to female *X. h. strigatus.* Female (40 mm.), on right above, exhibits *spotted* (Sp) pattern from *cortezi* with reduced expressivity. See Table VI, no. 89.

PLATE VII

FIG. 15. *X. m. cortezi* × *X. h. strigatus*. P_1 female, on right, middle, exhibits *spotted caudal* (Sc) pattern, which F_1 female (40 mm.), above, exhibits in an enhanced form. P_1 male, on left, below. See Table VI, no. 99. (h26).

FIG. 16. Second backcross offspring of h26 to male *X. h. strigatus*. Female (50 mm.) exhibits melanotic caudal fin and peduncle with an overgrowth. Note slashes of pigment in dorsal fin and several melanophore clusters on body, at least one of which is located anterior to the dorsal fin. See Table VI, no. 103.

FIG. 17. Cleared skin from side of *cortezi-hellerii* Sc hybrid (353) showing macromelanophore cluster and faint mid-lateral stripe. Anterior to the right. About 15½ ×.

FIG. 18. Cleared skin from side of wild male *X. v. variatus* showing reticulum, *spotted* pattern and vertical bar (at center). Anterior to the right. About 14¾ ×.

PLATE VIII

FIG. 19. *X. h. guentheri* from the Belize River in British Honduras, female above, male below. Both fish exhibit the *spotted* (Sp) pattern.

FIG. 20. Hybrid *X. h. guentheri* × *X. maculatus*. Fish on the right exhibits *spotted* (Sp) pattern from *guentheri*. Fish (50 mm.), on the left, exhibits a peculiar, undiagnosed atypical growth not associated with Sp. See Table VI, no. 119. (320).

FIG. 21. Backcross offspring of 320 to male *X. maculatus*. Male exhibits *spotted* (Sp) pattern from *guentheri* and nearly completely suppressed *striped* (Sr) pattern from *maculatus*. See Table VI, nos. 40, 127.

FIG. 22. Inbred, second backcross offspring of 320 to female *X. maculatus*. Female (28 mm.), exhibits *spotted* (Sp) pattern from *guentheri* and *striped* (Sr) pattern from *maculatus*. See Table VI, nos. 52, 126.